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EFFECT OF THYROXINE ON THE DIFFERENT BLOOD PICTURES OF MULBERRY SILKWORM, *Bombyx mori* L.

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ABSTRACT: *Effect of feeding last instar larvae of mulberry silkworm Bombyx mori L. by leaves dipped in thyroxine hormone at 2.5 ppm was studied to determine the changes in blood picture as, blood volume (BV), Total haemocyte counts (THC), differential haemocyte counts (DHC) and absolute differential haemocyte counts (ADHC). Results showed that larvae fed on thyroxine presented an increase in BV during the feeding period, until early spinning and during prepupal stage. THC and AHC showed an increase in treated larvae during the different developmental periods of the last larval instar. Daily changes of five haemocyte types were investigated as, prohaemocytes, plasmatocytes, coagulocytes, spherules and oenocytoides. Differential and absolute differential haemocyte counts showed changes and fluctuation during the different developmental periods of the last larval instar. Prohaemocytes showed an increase of DHC and ADHC during feeding and spinning period of treated larvae. Thyroxine improve silk production by increasing the DHC and the ADHC of spherule cells during spinning period in addition to enhancing the immune response of treated larvae by increasing the ADHC of plasmatocytes and coagulocytes during the most developmental periods. Oenocytoides showed their maximum increase of DHC and ADHC during the feeding period of treated larvae and during the end of spinning period and prepupal stage.*

Keywords: *Bombyx mori, Thyroxine, Haemocytes, Blood Volume, Silk Production.*

INTRODUCTION

Mulberry silkworm is the most important sources of natural silk in the world. Recently, much research has been done on the diet supplementation of mulberry leaves fed to silkworms. These supplementations include hormones such as thyroxine, which could be used to increase the cocoon and silk production (Thyagaraja *et al.*, 1991 and Saad, 2009). One of the very important subjects which should be studied when silk production increases is the changes occurring in the blood picture, blood volume and haemocytes are two of the very important factors in blood picture (Essawy *et al.*, 1999). Insect haemocytes, like vertebrate leucocytes, are a mixture of cell types with different morphological and biological functions. Haemocytes have the ability to discriminate stranger agents, mediate phagocytosis, cytotoxicity, encapsulation, wound repair and coagulation. These defense reactions were observed against pathogens, parasites and other foreign bodies, which entered in the haemocoel (Lackie, 1986 and Sanjayan *et al.,* 1996). Our knowledge of insect haemocytes and their functions in general is quite extensive (Jones, 1962; Arnold, 1979 and Beeman *et.al.* 1983). As a new trend workers are now trying to study the changes in the haemolymph. Any changes in total haemocyte Counts of particular insect directly or indirectly affect the insect adversely. Haemocytes perform various physiological functions in the body of insect. They direct nutrients to various tissues and store them. They perform phagocytosis, encapsulation of foreign bodies in the insect body cavity, coagulation to prevent loss of blood, nodule formation, transport of food materials, hormones and detoxification of metabolites and biological active materials (Patton, 1961). Five haemocyte types were identified in the haemolymph of Lepidopterous larvae: prohemocytes, plasmatocytes, granulocytes (Coagulocytes), spherulocytes and oenocytoides (Jones, 1962; Akai and Sato, 1973; Essawy *et al.,* 1985; Saad, 1996; Essawy *et al.,* 1999 and Saad, 2005). In present study effect of thyroxine on blood volume, total and deferential haemocyte counts have been studied.

MATERIALS AND METHODS

A: Insects:

Local hybrid (A422) of mulberry silkworm Larvae were reared under the laboratory hygrothermic conditions of 25±5^{0}C and 65-85% R.H., according to the conventional method in trays and were provided with suitable amounts of fresh mulberry leaves. All larvae which molted to the last larval instar at the same time were grouped and used in the experiments. The different developmental periods of the last larval instar of mulberry silkworm were classified according to (Calvez, 1980; Essawy, 1991 and saad, 1996). Feeding period (begin from 1st day until 8th day), early spinning period (at 9th day), spinning period (at 10th day) end of spinning period (at 11th day) and finally prepupal stage at twelve day.

B: Chemical:

Thyroxine, or 3:5, 3:5 tetra iodothronine (T4). Thyroxine was obtained from SIGMA company as L– thyroxine (3–(4–(4–hydroxy–3, 5–diiodophenoxy)-3, 5–diiodophenyl)–l-alanine-.T4). Stock solution of 25 ppm thyroxine was prepared by dissolving 25 mg. thyroxine in 100 ml sodium hydroxide 0.1 N and 900 ml distilled water. Clean mulberry leaves were divided into two groups, the first one were dipped in distilled water, the second group were dipped in 2.5 ppm of thyroxine solution for 10 minutes then left to dray to obtain the untreated and treated leaves which introduced to 60 larvae for each of check and treatment.

C: Methods of testing the parameters.

C-1: Blood volume (BV):

Daily blood weight was recorded by cutting prolegs of larvae, and then the haemolymph was collected to last drop. Blood volume was calculated by microliter (µL) after measuring of blood weight and blood density of tested larvae, (Arniold and hinks, 1976).

C-2: Total haemocyte counts (THC):

Total haemocyte counts were made after larvae were killed in a hot-water bath (56-58 °C). Haemolymph was withdrawn by cutting a proleg with fine scissors. Blood volume was quickly drawn into a Thoma white blood pipette and diluted with 2% versene plus a trace of methylene blue or crystal violet (Wittig, 1966; Shapiro, 1968 and Essawy, 1991). Pipette was shaken gently for several minutes, and transferred to a Nauber double lined haemocytometer and haemocytes were counted in squares situated at the four corners of the caliberated area. THC was calculated according to Jones, (1962). Absolute haemocyte counts (AHC) can be calculated from THC and BV according to (Shapiro, 1968; Arnold and Hinks, 1976; and Essawy, 1991).

C-3: Differential haemocyte counts (DHC):

Differential haemocyte counts were made after the larvae were killed in a hot-water bath (56-58 °C). Blood was withdrawn by cutting a proleg of larvae with a fine scissor. A drop of haemolymph was smeared on glass slide, (Shapiro, 1968 and Essawy, 1985). Smears were allowed to dry and were stained by Giemsa and Wright's blood stain according to (Shapiro, 1968; Arnold and Hinks, 1976; and Essawy, 1990). Smears were examined under oil immersion, and the haemocytes were differentiated using the classifications of (Jones, 1962; Akai and Sato, 1973 and Essawy *et al.,* 1985). Absolute differential haemocyte counts (ADHC) can be calculated from AHC and DHC.

RESULTS AND DISCUSSION

Effect of thyroxine on different biological aspects and silk production of mulberry silkworm *Bombyx mori* has been investigated in previous paper Saad, (2009). Last instar larvae fed on mulberry leaves was dipped in thyroxine solution at concentrations of 0.025, 0.125, 0.25, 1.25, 2.50, 5 and 10 ppm. Results indicated that the highly and significant values of the mean weight of mature larva, silk gland, pupa, fresh cocoon, and cocoon shell was gained by larvae fed on mulberry leaves dipped in 2.5 ppm of thyroxine. In current study the effect of 2.5 ppm of thyroxine was examined on the blood picture of the last larval instar of mulberry silkworm as follow:

1. Effect of Thyroxine on Blood Volume:

Blood volume presented gradual increase from the beginning of last larval instar until reached to maximum peak 561±69 and 614±20 µL in both untreated and treated larvae respectively at 9th day with an increase about 9.5% in treated larvae, then decreased until reached to minimum values 204±69 and 296±38 µL in both untreated and treated respectively, with an increase about 45.1% in treated larvae, (Table 1 and Fig. 1). The gradual increase of blood volume was positively corresponding to the feeding period. While the decrease from 9th day (the early spinning) until the prepupal stage at twelve day was according to the end of feeding in which larvae evacuated their midgut contents. These results are agreed with those reported by Saad, (1996 and 2005) and Essawy *et al.*, (1999).

Table 1: Daily changes in blood volume, haemocyte counts of mulberry silkworm larvae fed on thyroxine.

Days	Blood volume (µL) ± sd		(THC) Mean no. of cells/mm^3± sd		(AHC) Mean no. of cells/larva	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
1	94 ± 20	101 ± 18	6033 ± 252	6411 ± 1159	567102	647511
2	117 ± 8	134 ± 18	6757 ± 316	7467 ± 1115	790569	1000578
3	124 ± 16	149 ± 21	10967±1002	11300 ± 900	1359908	1683700
4	153 ± 20	222 ± 30	11947 ± 952	12477 ± 1272	1827891	2769894
5	268 ± 37	332 ± 75	12560±1057	13700 ± 2022	3366080	4548400
6	351 ± 33	363 ± 65	14370±1045	17033 ± 1365	5043870	6182979
7	407 ± 93	414 ± 68	16553±1350	18467 ± 685	6737071	7645338
8	410 ± 78	462 ± 70	18180±457	19364 ± 704	7453800	8946168
9	561 ± 69	614 ± 20	21385±1000	23545 ± 1269	11996985	14456630
10	399 ± 112	410 ± 66	17163±808	17873 ± 1415	6848037	7327930
11	295 ± 127	388 ± 59	14433±513	14983 ± 1200	4257735	5813404
12	204 ± 69	296 ± 38	10853±301	11224 ± 1062	2214012	3322304

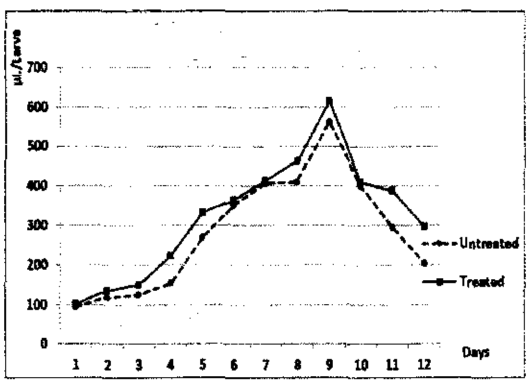

Fig. (1): Daily changes in blood volume of mulberry silkworm fed on thyroxine during the last larval instar.

2. Effect of Thyroxine on the Haemocyte Counts:

THC of untreated and treated larvae showed gradual increase from the first day until reached to maximum peak at 9^{th} day, the early of spinning period with an increase about 10.1% in treated larvae as showed in (Table 1 and Fig. 2). From 9^{th} day THC showed gradual decrease until prepupal stage. These results are resembled to those reported by Essawy, (1991); Essawy *et al.*, (1999) and Saad, (2005). AHC showed the same trend of THC. Treated larvae showed the maximum peak of AHC at 9^{th} day with an increase about 20.5% higher than untreated then decreased until prepupal stage, as showed in (Table 1 and Fig. 3). These findings agreed with those of Saad, (1996 and 2005) and accordance with those of Essawy, (1999).

These results showed a positive correlation between BV, THC and AHC in both untreated and treated larvae during the feeding period from the 1^{st} day until 9^{th} day (the end of feeding period and beginning of spinning). These findings resemble to those of Essawy *et al.*, (1999). An increase of BV, THC and AHC after treatment could be attributed to the indirect effect on the hormonal balance which in turn affects the BV and THC. Sanjayan *et al.*, (1996) reported that AHC in circulation depends on both THC and BV, while Nittono, (1960) advocated an inverse relationship between THC and BV in *B. mori* larvae.

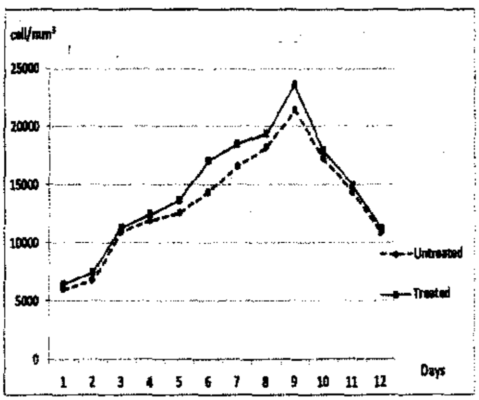

Fig. (2): Daily changes in total haemocyte counts (THC) of mulberry silkworm fed on thyroxine during the last larval instar.

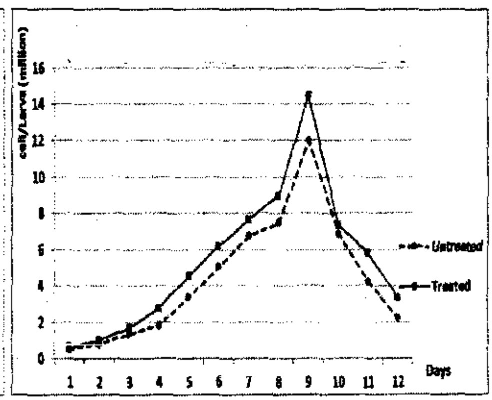

Fig.(3): Daily changes in absolute haemocyte counts (AHC) of mulberry silkworm fed on thyroxine during the last larval instar.

3. Effect of Thyroxine on the Differential Haemocyte Counts:

Five haemocyte types: Prohaemocytes, Plasmatocytes, Coagulocytes, Spherules and Oenocytoides were recorded during the last larval instar.

3.1. Effect on Prohaemocytes:

Prohaemocytes are round or oval cells, characterized by a low concentration of intercellular organelles. Prohaemocytes ranged from 3.3-7.3% and 4.3-9.3% of circulating haemocytes in untreated and treated larvae respectively. Untreated and treated larvae showed a fluctuation trend in DHC of prohaemocyte type during the last larval instar. Treated larvae presented a positive increase in DHC about 75.5, 38.1 and 25% during the 3rd, 4th and 7th days of feeding period. While a decreases about 17.5 and 60.6% were observed at 8th and 9th days, respectively. During spinning period treated larvae showed an increase about 60.6 and 17.5% in DHC at 10th and 11th days respectively, (Table 2 and Fig. 4).

ADHC of treated larvae showed gradual increase during the feeding period until reached to maximum peak (4.266 cell/Larvae x 10^6) at 7th day higher than untreated (2.695 cell/Larvae x 10^6) by 58.3%, then decreased sharply at 8th day to meet the same value of untreated at 9th day (Table 3 and Fig. 5). While during the spinning period at 10th day and until the prepupal stage at twelve day ADHC was increased in treated larvae than untreated one by 71.9, 60.5 and 61.3% respectively. Our results are in partial agreement with those of Saad, (2005) who observed fluctuation trend and positive effect in ADHC after fed of mulberry silkworm with biosalt.

Table 2: Daily changes in differential haemocyte counts of mulberry silkworm fed on thyroxine during the last larval instar.

Days	% DHC Means ± sd									
	Prohaemocytes		Plasmatocytes		Coagulocytes		Spherules		Oenocytoides	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
1	6.0±1.0	5.3±0.6	27.3±2.5	33.7±3.5	60.7±3.1	58.3 ± 5.7	3.0± 1.0	2.0± 1.0	1.7± 0.6	1.3 ± 0.6
2	7.3±1.5	7.7±1.5	28.3±2.5	35.0±5.0	59.0±3.6	52.7 ± 6.4	1.7± 0.6	1.3± 0.6	2.3± 0.6	1.3 ± 1.0
3	5.3±0.6	9.3±0.6	41.0±1.0	38.0±6.3	51.0±1.7	48.0 ± 6.0	1.7± 0.6	2.7± 0.6	2.3± 0.6	2.0 ± 1.0
4	6.3±1.2	8.7±3.5	34.0±12.2	36.0±3.6	52.3±7.8	49.0 ± 1.0	1.6± 1.2	2.3± 0.6	2.3± 0.6	2.7 ± 0.6
5	6.3±1.5	6.3±3.5	37.7±10.8	41.7±7.6	52.7± 9.5	48.0± 10.7	1.7± 0.6	1.7± 0.6	1.7± 0.6	2.0 ± 0.0
6	3.3±0.6	4.0±1.0	35.7±10.2	33.3±2.9	57.3± 9.3	57.7± 2.5	2.7± 1.2	2.7± 0.6	1.0± 0.0	2.3 ± 0.6
7	4 ± 1.0	5.0±1.7	40.7±4.0	32.0±2.7	51.0± 5.0	58.7± 2.3	2.3± 1.5	2.3± 1.2	1.3± 0.6	2.0 ± 0.0
8	4.7±2.3	4.0±1.0	30.3±1.5	33.3±7.6	61.7± 1.5	60.7± 7.4	1.0± 0.0	1.3± 0.6	2.3± 0.6	1.0 ± 0.0
9	5.7±2.1	4.7±2.9	28.7±5.8	24.0±5.3	62.0± 7.2	68.0± 4.4	1.7± 0.6	1.3± 0.6	2.0± 1.0	1.3 ± 0.6
10	3.3±1.5	5.3±1.5	23 ± 1.0	31.0±5.3	70.3± 2.5	59.7± 4.0	1.7± 0.6	2.7± 2.1	1.7± 0.6	1.3 ± 0.6
11	4.0±0.0	4.7±1.5	23.7±1.5	30.3±8.5	68.3± 1.5	61.3± 9.9	2.3± 0.6	1.7± 0.6	1.7± 0.6	2.3 ± 0.6
12	4.0±1.0	4.3±1.2	36.0±13.5	25.0±5.0	55.7±12.1	66.0± 3.6	2.7± 2.1	2.0± 0.0	1.7± 0.6	2.7 ± 0.6

Table 3: Daily changes in absolute differential haemocyte counts of mulberry silkworm fed on thyroxine during the last larval instar.

Days	ADHC cell no. x 10^6 /Larvae									
	Prohaemocytes		Plasmatocytes		Coagulocytes		Spherules		Oenocytoides	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
1	0.340	0.343	1.548	2.182	3.442	3.775	0.170	0.130	0.096	0.084
2	0.577	0.771	2.237	3.502	4.664	5.273	0.134	0.130	0.182	0.130
3	0.721	1.566	5.576	6.398	6.936	8.082	0.231	0.455	0.313	0.337
4	1.152	2.410	6.215	9.972	9.560	13.573	0.293	0.637	0.420	0.748
5	2.121	2.866	12.555	18.967	17.739	21.832	0.572	0.773	0.572	0.910
6	1.665	2.473	18.007	20.589	28.901	35.676	1.362	1.669	0.504	1.422
7	2.695	4.266	27.420	27.302	34.359	50.081	1.550	1.962	0.876	1.706
8	3.503	3.207	22.585	26.696	45.999	48.661	0.745	1.042	1.714	0.802
9	6.838	6.795	34.431	34.696	74.381	98.305	2.040	1.879	2.399	1.879
10	2.260	3.884	15.751	22.717	48.142	43.748	1.164	1.979	1.164	0.953
11	1.703	2.733	10.091	17.615	29.080	35.636	0.979	0.988	0.724	1.337
12	0.886	1.429	7.970	8.306	12.332	21.927	0.598	0.665	0.376	0.897

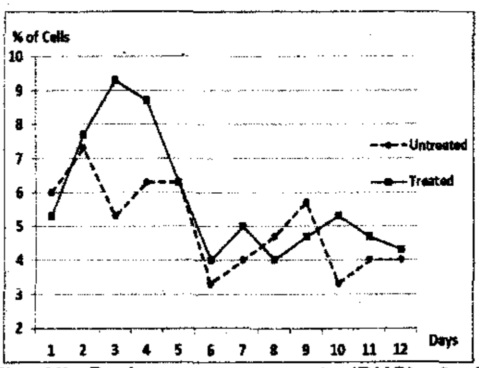

Fig. (4): Prohaemocyte counts (DHC) of mulberry silkworm fed on thyroxine during the last larval instar.

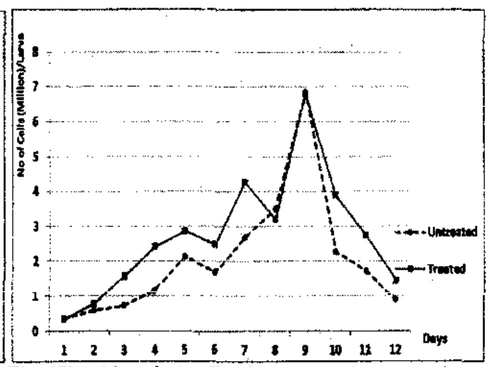

Fig.(5): Absolute Prohaemocyte counts (ADHC) of mulberry silkworm fed on thyroxine during the last larval instar.

3.2. Effect on Plasmatocytes:

Plasmatocytes are characterized by an elongated nucleus which contains a round nucleolus and scattered chromatin masses. Cytoplasm contains granular endoplasmic reticulum and golgi complexes. Plasmatocytes may be subdivided into 3 types: thick, medium and thin according to (Nittono, 1960). Plasmatocytes ranged from 23-41% and 24-41.7% of circulating haemocytes in both untreated and treated larvae, respectively. As showed in Table (2) and Fig. (6) DHC fluctuated during the last larval instar in both untreated and treated larvae. DHC of treated larvae increased than those of untreated by 23.4% at 1st day, and then decreased at 3rd day to increase again at 5th day by 11.8%. From 5th day and until the prepupal stage DHC of treated larvae showed decreases except an increase about 1% was observed during the mature larvae stage at 8th day and an increases about 34.8 and 27.9% during spinning period at 10th and 11th days respectively.

ADHC of treated larvae was increased than those of untreated by 60.5, 15.1, 14.3 and 18.2% during the 4th, 5th, 6th and 8th days of feeding period respectively. Other increase in ADHC was observed during spinning period was higher than chick by 44.2 and 74.6% at 10th and 11th days respectively, (Table 3 and Fig. 7). The results were in partial agreement with those of (Saad, 1996 and Essawy *et al*, 1999 in Eri-silkworm and Saad, 2005 in *B. mori*). ADHC's of plasmatocytes were greater in treated larvae than untreated in most developmental periods which suggests that the immune response of treated larvae is more than untreated. (Sanjayan *et al.*, 1996).

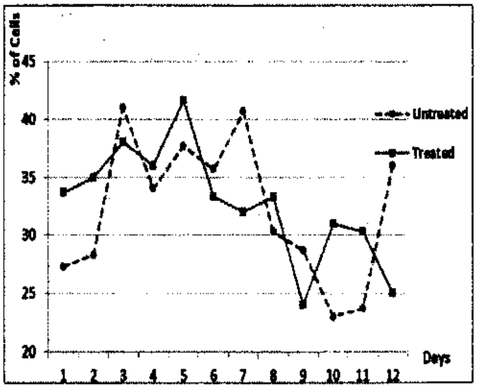

Fig. (6): Plasmatocyte counts (DHC) of mulberry silkworm fed on thyroxine during the last larval instar.

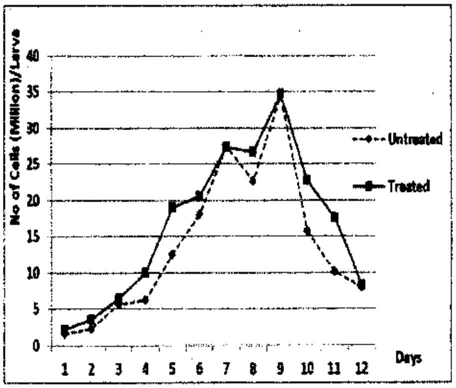

Fig.(7): Absolute Plasmatocyte counts (ADHC) of mulberry silkworm fed on thyroxine during the last larval instar.

3.3. Effect on Coagulocytes:

Coagulocytes (granulocytes) are recognized as spherical or oval cells which vary considerably in size. Their nuclei are relatively small, and the cytoplasm contains numerous cytoplasmic inclusions. Under the light microscope, two types of granular were found, different in size, shape, and in their cytoplasmic inclusions and vacuoles. Coagulocytes ranged from 51-70.3% and 48-68% in untreated and treated larvae respectively. As showed in

Table (2) and Fig. (8) DHC of treated larvae was decreased than untreated during the feeding period except that increase of 13.7% at 7th day and those of 9.7% at 9th day. Other increase about 18.5% was observed during prepupal stage at twelve day of treated larvae. DHC of total coagulocytes presented its maximum peak at twelve day higher than control in Eri-silkworm 5th instar larvae treated with IGR, this increase seems to be true as the BV decrease at this time (Saad, 1996 and Essawy *et al*, 1999).

Treated larvae showed a semi gradual increase in ADHC during the feeding period from the 1st day until the the 9th day of feeding period. The maximum increase of ADHC was observed higher than untreated by 45.8 and 32.2% at 7th and 9th days respectively, during the feeding period. Other increase about 77.8% was also observed at twelve day during the prepupal stage of treated larvae, (Table 3 and Fig. 9). Present results are similar to the finding of Saad, (1996) and Essawy *et al*, (1999) in Eri-silkworm and Saad, (2005) in mulberry silkworm. As showed in Fig. (9) ADHC of coagulocytes was greater in treated larvae than untreated in most developmental periods as plasmatocytes which suggests that the immune response of treated larvae is more than untreated. (Sanjayan *et al.*, 1996).

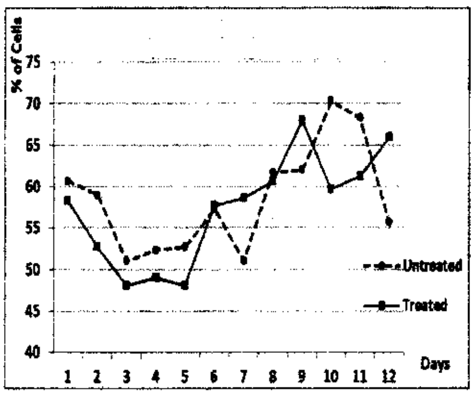

Fig. (8): Coagulocyte counts (DHC) of mulberry silkworm fed on thyroxine during the last larval instar.

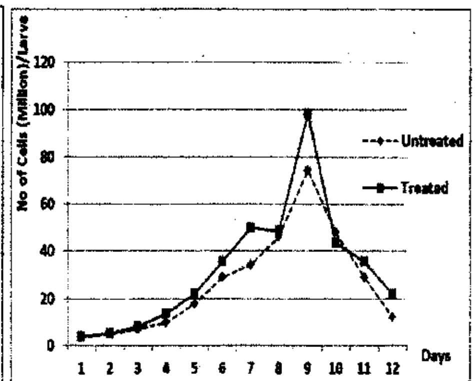

Fig.(9): Absolute Coagulocyte counts (ADHC) of mulberry silkworm fed on thyroxine during the last larval instar.

3.4. Effect on Spherules:

Spherule cells are oval and contain many characteristic spherules in their cytoplasm, the nucleus is usually small and contains a nucleolus and chromatin bodies, the cytoplasm contain many small spherules, which appear dark in phase-contrast microscopy. Spherules ranged from 1-3% and 1.3-2.7% in untreated and treated larvae. DHC of treated larvae begin and finished with a low value in comparison to untreated, as showed in Table (2) and Fig. (10). DHC of treated larvae increased than untreated by 58.8, 43.8 and 30% respectively at 3rd, 4th and 8th days of feeding period. DHC presented also the maximum increase higher than untreated by 58.8% during the peak of spinning period at 10th day, this results are resemble to those reported by (Saad, 1996 and Essawy *et al*, 1999) in Eri-silkworm and (Saad, 2005) in mulberry silkworm.

ADHC of treated larvae increased than untreated by about 97, 117.4, 35.1, 22.5, 26.6 and 39.9% during the feeding period from 3rd day until 8th days respectively. Other increase about 70% of ADHC was observed at 10th day during the peak of spinning period, (Table 3 and Fig. 11). Saad, (2005) revealed the same trend in *B. mori* last larval instar which showed the maximum peak of ADHC at 10th day. Nittono, (1960) examined the larval haemocytes of 301 silkworm strains, in 26 strains, spherule cells were not present and concluded that those strains produced less silk than those strains containing spherule cells. Akai and Sato, (1979) investigated that spherule cells were noted to play a role in the secretion of some haemolymph protein. Moreover, spherule cells attributed to silk production (Nittono, 1960 and Essawy *et al*, 1997). Essawy *et al.*, (1999) reported that spherule cells played a significant role in silk production. In present study the maximum value of ADHC of spherule cells in larvae treated with thyroxine correspond to the maximum value of the DHC observed during the peak of spinning of cocoon at 10th day of treated larvae with an increase about 58.8 and 70% in DHC and ADHC respectively, this might explain the effect of thyroxin on enhanced silk production.

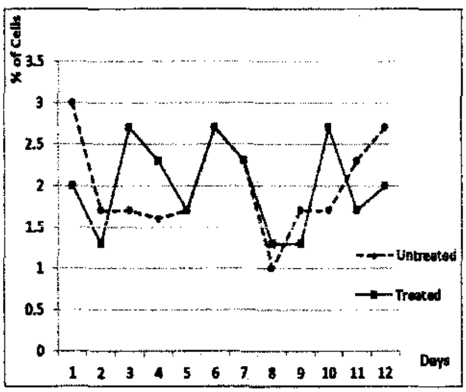

Fig.(10): Spherule counts (DHC) of mulberry silkworm fed on thyroxine during the last larval instar.

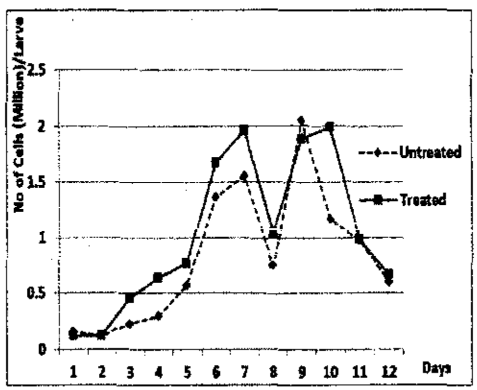

Fig.(11): Absolute Spherule counts (ADHC) of mulberry silkworm fed on thyroxine during the last larval instar.

3.5. Effect on Oenocytoides:

Oenocytoides are large, spherical cells characterized by large cytoplasmic inclusions. The surrounding cytoplasm is filled with numerous ribosomes and bundles of fine fibers, but the endoplasmic reticulum is poorly developed at this stage. Oenocytoides ranged from 1-2.3% in untreated and 1-2.7% in treated larvae. As showed in (Table 2 and Fig. 12) DHC of treated larvae was observed with a low level during the first three days until reached to first peak (2.7% ± 0.6) at 4th day higher than those of untreated (2.3% ± 0.6) by 17.4%. The second peak (2.3% ± 0.6) was observed at 6th day raised than those of untreated (1.0% ± 0.0) by 130%. DHC of treated larvae showed a sharp decrease than untreated at 8th day by 130%, and during the spinning period. While an increase about 35.3 and 58.8% was observed in treated larvae at the end of spinning in 11th day and during prepupal stage respectively. Oenocytoides usually decrease after pupation in *B. mori* (Nittono, 1960).

ADHC of treated larvae showed gradual increases about (78.1, 59.1, 182.1 and 94.8%) during the 4th, 5th, 6th and 7th day of feeding period. While, sharp decrease about 113.9% was observed at 8th day of feeding period followed by another decreases about 27.7 and 22.2% at 9th and 10th days, respectively. ADHC of treated larvae was increased than untreated one by (84.7 and 138.3%) at 11th day, the end of spinning and during the prepupal stage respectively, (Table 3 and Fig. 13).

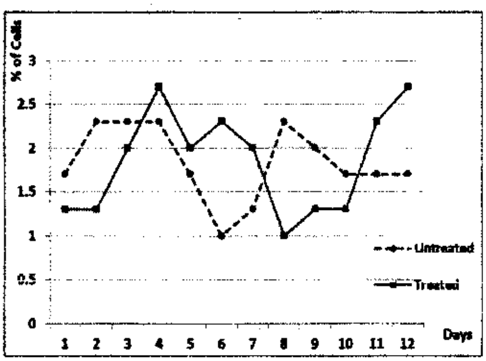

Fig.(12): Oenocytoide counts (DHC) of mulberry silkworm fed on thyroxine during the last larval instar.

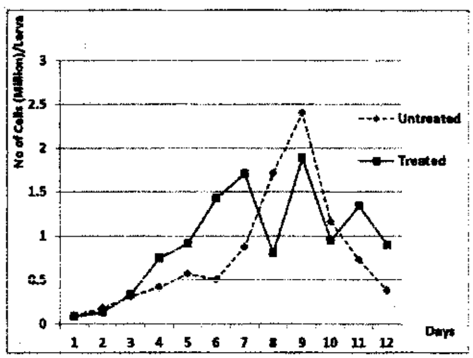

Fig.(13): Absolute Oenocytoide counts (ADHC) of mulberry silkworm fed on thyroxine during the last larval instar.

In present study thyroxine hormone caused a positive changes in BV, THC and AHC during the feeding period. It is well established that changes in DHC during insect development are common (Arnold, 1974 and Essawy, 1985) and appear to be under endocrine control (Judy and Marks, 1971;

Takeda, 1977; Essawy, 1985 and Essawy *et al.,* 1997). Thyroxine hormone affect positively the hormonal balance which might be affect indirectly the proportion of the main haemocyte types were in positive relation with blood volume. Changes in the number of circulating haemocytes might be associated with changes in blood volume (Shapiro, 1979). From the current results it was observed that DHC and ADHC of prohaemocytes, plasmatocytes and spherule cells was greater in treated larvae with thyroxine than untreated one during the spinning period, while coagulocytes and oenocytoides were less than untreated. It has been investigated the role of spherule cells in secretion of some haemolymph protein (Akai and Sato, 1979) and in silk production (Nittono, 1960 and Essawy *et al*, 1997). Yamashita and Iwabuchi, (2001) investigated that over 60% of the non-dividing prohemocytes differentiated to plasmatocytes or granulocytes. Some of the granulocytes subsequently transformed to spherulocytes, 59.2% of the dividing prohemocytes, the daughter cells differentiated into other types of haemocytes such as plasmatocytes, granulocytes and spherulocytes, and the remainder divided into new prohaemocytes. Current results showed that ADHC of plasmatocytes and coagulocytes was greater in treated larvae than untreated, this might explain that thyroxine affected the immune response of silkworm larvae, according to Sanjayan *et al.,* (1996) who investigated that each developmental stages of larvae is character-ized by specific haemocyte composition to meet the developmental needs, because the effective physiological mechanisms of phagocytosis, encapsulation, and other related defense mechanisms are primarily due to the avilability of circulatory immune cells particularly plasmatocytes and granulocytes.

In conclusion, oral uptake of 2.5 ppm of thyroxine not only enhanced the different biological characters as mean weight of mature larva, silk gland, pupa, fresh cocoon, and cocoon shell, (Saad, 2009) but also enhanced the physiological aspects in present study. Thyroxine increased the BV during the feeding period, the beginning and end of spinning period as well as prepupal stage. Thyroxine increased the THC during all different periods and enhanced the AHC. Thyroxine affected also the DHC and ADHC during the developmental periods of the last larval instar specially spherule cells which increased during the peak of spinning period. This might explain the effect of thyroxin on enhanced silk production by affecting this type of haemocytes which played a significant role in silk production in addition to enhancing the immune response by increase the absolute numbers of plasmatocytes and granulocytes in treated larvae. The larvae treated with thyroxine from a box of egg weighted 20 gm coasted 25 pounds to give us 2.8 kg over than untreated one by an increase about 125 pounds, so the thyroxine treatments in a mass rearing for commercial production is more significant.

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تأثير هرمون الثيروكسين على صور الدم المختلفة لدودة الحرير التوتية

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الملخص العربي

تم دراسة تأثير تغذية يرقات العمر الخامس لدوده الحرير التوتية بأوراق التوت المعاملة بهرمون الثيروكسين ٢,٥ جزء في المليون على تغيرات صوره الدم مثل حجم الدم وعدد خلايا الدم وكذلك على النسبة المؤيه والعدد الكلى لأنواع خلايا الدم المختلفة. وقد أوضحت النتائج زياده متوسط حجم الدم في اليرقات التى تغذت على الثيروكسين خلال فتره التغذية وحتى بداية غزل الشرنقة وخلال طور ما قبل العذراء. زياده عدد خلايا الدم فى المليمتر المكعب وكذا العدد المطلق بكل يرقه خلال فترات نمو العمر اليرقى الاخير مقارنه بالكنترول. تم دراسة التغيرات اليومية لخمسه انواع من خلايا الدم هى: (prohaemocytes, plasmatocytes, coagulocytes, spherules, oenocytoides) حيث أظهرت الدراسة تغيرات وتذبذب فى النسبة المؤيه لخلايا الدم وكذلك العدد المطلق لها خلال العمر اليرقى الاخير. فقد زادت النسبة المؤيه والعدد المطلق لخلايا الدم من النوع Prohaemocytes خلال فتره التغذية وفتره غزل الشرنقة باليرقات المعاملة. أدى الثيروكسين الى تحسن إنتاج الحرير عن طريق زياده النسبة المؤيه والعدد المطلق لخلايا النوع spherule خلال مرحله غزل الشرنقة. علاوة على تأثيره الإيجابى على مناعة اليرقات من خلال زياده العدد المطلق لخلايا النوعين plasmatocyte, coagulocyte خلال معظم فترات النمو المختلفة. أما خلايا النوع Oenocytoide فقد أدت المعاملة بالثيروكسين إلى زياده النسبة المؤيه والعدد المطلق لها خلال فتره التغذية وبعد انتهاء غزل الشرنقة وكذلك خلال طور ما قبل العذراء.